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(54) Bacterium producing L-glutamic acid and method for producing L-glutamic acid

(57) L-Glutamic acid is produced by culturing a coryneform bacterium having L-glutamic acid producing ability, in which trehalose synthesis ability is decreased or deleted by, for example, disrupting a gene coding for trehalose-6-phosphate synthase, a gene coding for maltooligosyltrehalose synthase, or both of these genes to produce and accumulate L-glutamic acid in the medium, and collecting the L-glutamic acid from the medium.

EP 1 174 508 A2

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Description

Background of the invention

Field of the Invention

[0001] The present invention relates to a novel L-glutamic acid producing bacterium and a method for producing L-glutamic acid by fermentation utilizing it. L-glutamic acid is an important amino acid as foodstuffs, drugs and so forth.

Description of the Related Art

[0002] Conventionally, L-glutamic acid is mainly produced by fermentative methods using so-called L-glutamic acid producing coryneform bacteria belonging to the genus *Brevibacterium, Corynebacterium* or *Microbacterium*, or mutant strains thereof (Amino Acid Fermentation, pp.195-215, Gakkai Shuppan Center, 1986).

[0003] It is known that, in the production of L-glutamic acid by fermentation, trehalose is also produced as a secondary product. Therefore, techniques have been developed for decomposing or metabolizing the produced trehalose. Such techniques include the method of producing an amino acid by fermentation using a coryneform bacterium in which proliferation ability on trehalose is induced (Japanese Patent Laid-open (Kokai) No. 5-276935) and the method of producing amino acid by fermentation using a coryneform bacterium in which a gene coding for trehalose catabolic enzyme is amplified (Korean Patent Publication (B1) No. 165836). However, it is not known how to suppress the formation of trehalose itself in an L-glutamic acid producing bacterium.

[0004] In *Escherichia coli*, the synthesis of trehalose is catalyzed by trehalose-6-phosphate synthase. This enzyme is known to be encoded by *otsA* gene. Further, it has been also reported that an *otsA* gene-disrupted strain of *Escherichia coli* can scarcely grow in a hyperosmotic medium (H.M. Glaever, *et al., J. Bacteriol., 170*(6), 2841-2849 (1998)). However, the relationship between disruption of *otsA* gene and production of substances has not been known.

[0005] On the other hand, although the *treY* gene is known for *Brevibacterium helvolum* among bacteria belonging to the genus *Brevibacterium* bacteria, any *otsA* gene is not known for them. As for bacteria belonging to the genus *Mycobacterium* bacteria, there is known a pathway via a reaction catalyzed by a product encoded by *treS* gene (trehalose synthase (TreS)), which gene is different from the *otsA* gene and *treY* gene, as a gene coding for a enzyme in trehalose biosynthesis pathway (De Smet K.A., *et al.*, Microbiology, *146* (1), 199-208 (2000)). However, this pathway utilizes maltose as a substrate and does not relate to usual L-glutamic acid fermentation that utilizes glucose, fructose or sucrose as a starting material.

SUMMARY OF THE INVENTION

[0006] An object of the present invention is to improve production efficiency of L-glutamic acid in L-glutamic acid production by fermentation using coryneform bacteria through suppression of the production of trehalose as a secondary product.

[0007] The inventors of the present invention assiduously studied in order to achieve the aforementioned object. As a result, they found that bacterium belonging to the genus *Brevibacterium* contained *otsA* gene and *treY* gene like *Mycobacterium tuberculosis*, and the production efficiency of L-glutamic acid was improved by disrupting at least one of these genes. Thus, they accomplished the present invention.

[0008] That is, the present invention provides the followings.

(1) A coryneform bacterium having L-glutamic acid producing ability, wherein trehalose synthesis ability is decreased or deleted in the bacterium.

(2) The coryneform bacteria according to (1), wherein the trehalose synthesis ability is decreased or deleted by introducing a mutation into a chromosomal gene coding for an enzyme in a trehalose synthesis pathway or disrupting the gene.

(3) The coryneform bacteria according to (2), wherein the gene coding for the enzyme in trehalose synthesis pathway consists of a gene coding for trehalose-6-phosphate synthase, a gene coding for maltooligosyltrehalose synthase, or both of these genes.

(4) The coryneform bacteria according to (3), wherein the gene coding for trehalose-6-phosphate synthase codes for the amino acid sequence of SEQ ID NO: 30, and the gene coding for maltooligosyltrehalose synthase codes for the amino acid sequence of SEQ ID NO: 32.

(5) A method for producing L-glutamic acid comprising culturing a coryneform bacterium according to any one of (1) to (4) in a medium to produce and accumulate L-glutamic acid in the medium, and collecting the L-glutamic acid from the medium.

(6) A DNA coding for a protein defined in the following (A) or (B):

(A) a protein having the amino acid sequence of SEQ ID NO: 30,
(B) a protein having an amino acid sequence of SEQ ID NO: 30 including substitution, deletion, insertion or addition of one or several amino acid residues and having trehalose-6-phosphate synthase activity.

(7) A DNA according to (6), which is a DNA defined in the following (a) or (b):

(a) a DNA containing a nucleotide sequence comprising at least the residues of nucleotide numbers 484-1938 in the nucleotide sequence of SEQ ID NO: 29,
(b) a DNA hybridizable with a nucleotide sequence comprising at least the residues of nucleotide numbers 484-1938 in the nucleotide sequence of SEQ ID NO: 29 under a stringent condition, showing homology of 55% or more to the foregoing nucleotide sequence, and coding for a protein having trehalose-6-phosphate synthase activity.

(8) A DNA coding for a protein defined in the following (A) or (B):

(A) a protein having the amino acid sequence of SEQ ID NO: 32,
(B) a protein having an amino acid sequence of SEQ ID NO: 32 including substitution, deletion, insertion or addition of one or several amino acid residues and having maltooligosyltrehalose synthase activity.

(9) A DNA according to (8), which is a DNA defined in the following (a) or (b):

(a) a DNA containing a nucleotide sequence comprising at least the residues of nucleotide numbers 82-2514 in the nucleotide sequence of SEQ ID NO: 31,
(b) a DNA hybridizable with a nucleotide sequence comprising at least the residues of nucleotide numbers 82-2514 in the nucleotide sequence of SEQ ID NO: 31 under a stringent condition, showing homology of 60% or more to the foregoing nucleotide sequence, and coding for a protein having maltooligosyltrehalose synthase activity.

[0009] The trehalose-6-phosphate synthase activity means an activity to catalyze a reaction in which α,α-trehalose-6-phosphate and UDP are produced from UDP-glucose and glucose-6-phosphate, and the maltooligosyltrehalose synthase activity means an activity to catalyze a reaction in which maltotriosyltrehalose is produced from maltopentose.

[0010] According to the present invention, production efficiency of L-glutamic acid in L-glutamic acid production by fermentation using coryneform bacteria can be improved through inhibition of the production of trehalose as a secondary product.

Preferreed Embodiments of the Invention

[0011] Hereafter, the present invention will be explained in detail.

[0012] The coryneform bacterium of the present invention is a coryneform bacterium having L-glutamic acid producing ability, in which trehalose synthesis ability is decreased or deleted.

[0013] The coryneform bacteria referred to in the present invention include the group of microorganisms defined in Bergey's Manual of Determinative Bacteriology, 8th edition, p.599 (1974), which are aerobic Gram-positive rods having no acid resistance and no spore-forming ability aerobic. They have hitherto been classified into the genus *Brevibacterium*, but united into the genus *Corynebacterium* at present *(Int. J. Syst. Bacteriol., 41,* 255 (1981)), and include bacteria belonging to the genus *Brevibacterium* or *Microbacterium* closely relative to the genus *Corynebacterium*. Examples of such coryneform bacteria are mentioned below.

Corynebacterium acetoacidophilum
Corynebacterium acetoglutamicum
Corynebacterium alkanolyticum
Corynebacterium callunae
Corynebacterium glutamicum
Corynebacterium lilium (Corynebacterium glutamicum)
Corynebacterium melassecola
Corynebacterium thermoaminogenes
Corynebacterium herculis

Brevibacterium divaricatum (Corynebacterium glutamicum)
Brevibacterium flavum (Corynebacterium glutamicum)
Brevibacterium immariophilum
Brevibacterium lactofermentum (Corynebacterium glutamicum)
Brevibacterium roseum
Brevibacterium saccharolyticum
Brevibacterium thiogenitalis
Brevibacterium ammoniagenes (Corynebacterium ammoniagenes)
Brevibacterium album
Brevibacterium cerium
Microbacterium ammoniaphilum

[0014] Specifically, the following strains can be exemplified.

Corynebacterium acetoacidophilum ATCC 13870
Corynebacterium acetoglutamicum ATCC 15806
Corynebacterium alkanolyticum ATCC21511
Corynebacterium callunae ATCC 15991
Corynebacterium glutamicum ATCC 13020, 13032, 13060
Corynebacterium lilium (Corynebacterium glutamicum) ATCC 15990
Corynebacterium melassecola ATCC 17965
Corynebacterium thermoaminogenes AJ12340 (FERM BP-1539)
Corynebacterium herculis ATCC13868
Brevibacterium divaricatum (Corynebacterium glutamicum) ATCC 14020
Brevibacterium flavum (Corynebacterium glutamicum) ATCC 13826, ATCC 14067
Brevibacterium immariophilum ATCC 14068
Brevibacterium lactofermentum (Corynebacterium glutamicum) ATCC 13665, ATCC 13869
Brevibacterium roseum ATCC 13825
Brevibacterium saccharolyticum ATCC 14066
Brevibacterium thiogenitalis ATCC 19240
Brevibacterium ammoniagenes (Corynebacterium ammoniagenes) ATCC 6871
Brevibacterium album ATCC 15111
Brevibacterium cerium ATCC 15112
Microbacterium ammoniaphilum ATCC 15354

[0015] The trehalose synthesis ability of such coryneform bacteria as mentioned above can be decreased or deleted by mutagenizing or disrupting a gene coding for an enzyme in trehalose synthesis pathway using mutagenesis treatment or genetic recombination technique. Such a mutation may be a mutation that suppresses transcription or translation of the gene coding for the enzyme in trehalose synthesis pathway, or a mutation that causes elimination or decrease of an enzyme in trehalose systhesis pathway. The enzyme in trehalose systhesis pathway may be exemplified by, for example, trehalose-6-phosphate synthase, maltooligosyltrehalose synthase, or both of these.

[0016] The disruption of a gene coding for an enzyme in trehalose systhesis pathway can be performed by gene substitution utilizing homologous recombination. A gene on a chromosome of a coryneform bacterium can be disrupted by transforming the coryneform bacterium with DNA containing a gene coding for an enzyme in trehalose systhesis pathway modified so that a part thereof should be deleted and hence the enzyme in trehalose systhesis pathway should not normally function (deletion type gene), and allowing recombination between the deletion type gene and a normal gene on the chromosome. Such gene disruption by homologous recombination has already been established. To this end, there can be mentioned a method utilizing a linear DNA or a cyclic DNA that does not replicate in coryneform bacteria and a method utilizing a plasmid containing a temperature sensitive replication origin. However, a method utilizing a cyclic DNA that does not replicate in coryneform bacteria or a plasmid containing a temperature sensitive replication origin is preferred.

[0017] The gene coding for an enzyme in trehalose systhesis pathway may be exemplified by, for example, the *otsA* gene or *treY* gene, or it may consist of both of these. Since the nucleotide sequences of the *otsA* gene and *treY* gene of *Brevibacterium lactofermentum* and flanking regions thereof have been elucidated by the present invention, those genes can be easily obtained by preparing primers based on the sequences and performing PCR (polymerase chain reaction, see White, T.J. *et al., Trends Genet., 5*, 185 (1989)) using the primers and chromosomal DNA of *Brevibacterium lactofermentum* as a template.

[0018] The nucleotide sequence comprising the *otsA* gene and the nucleotide sequence comprising the *treY* gene

of *Brevibacterium lactofermentum* obtained in the examples described later are shown in SEQ ID NOS: 29 and 31, respectively. Further, the amino acid sequences encoded by these nucleotide sequences are shown in SEQ ID NOS: 30 and 32, respectively.

[0019] The otsA gene and *treY* gene each may be one coding for a protein including substitution, deletion, insertion or addition of one or several amino acids at one or a plurality of positions, provided that the activity of trehalose-6-phosphate synthase or maltooligosyltrehalose synthase encoded thereby is not deteriorated. While the number of "several" amino acids differs depending on positions or types of amino acid residues in the three-dimensional structure of the protein, it is preferably 1-40, more preferably 1-20, further preferably 1-10.

[0020] A DNA coding for the substantially same protein as trehalose-6-phosphate synthase or maltooligosyltrehalose synthase described above can be obtained by, for example, modifying each of the nucleotide sequences by, for example, the site-directed mutagenesis method so that one or more amino acid residues at a specified site should involve substitution, deletion, insertion, addition or inversion. Such a DNA modified as described above may also be obtained by a conventionally known mutation treatment. The mutation treatment includes a method of treating DNA coding for trehalose-6-phosphate synthase or maltooligosyltrehalose *in vitro*, for example, with hydroxylamine, and a method for treating a microorganism, for example, a bacterium belonging to the genus *Escherichia* harboring a DNA coding for trehalose-6-phosphate synthase or maltooligosyltrehalose with ultraviolet irradiation or a mutating agent usually used for mutation treatment such as N-methyl-N'-nitro-N-nitrosoguanidine (NTG) and nitrous acid.

[0021] The substitution, deletion, insertion, addition, or inversion of nucleotide as described above also includes a naturally occurring mutant or variant on the basis of, for example, individual difference or difference in species or genus of microorganisms that harbor trehalose-6-phosphate synthase or maltooligosyltrehalose.

[0022] A DNA coding for the substantially same protein as trehalose-6-phosphate synthase or maltooligosyltrehalose synthase described above can be obtained by expressing such a DNA having a mutation as described above in a suitable cell, and examining the trehalose-6-phosphate synthase activity or maltooligosyltrehalose synthase activity of the expression product.

[0023] A DNA coding for substantially the same protein as trehalose-6-phosphate synthase can also be obtained by isolating a DNA hybridizable with a DNA having, for example, a nucleotide sequence corresponding to nucleotide numbers of 484-1938 of the nucleotide sequence shown in SEQ ID NO: 29 or a probe that can be prepared from the nucleotide sequence under a stringent condition, showing homology of 55% or more, preferably 65% or more, more preferably 75% or more, to the foregoing nucleotide sequence, and having trehalose-6-phosphate synthase activity from a DNA coding for trehalose-6-phosphate synthase having a mutation or from a cell harboring it. Similarly, a DNA coding for substantially the same protein as maltooligosyltrehalose synthase can also be obtained by isolating a DNA hybridizable with a DNA having, for example, a nucleotide sequence corresponding to nucleotide numbers of 82-2514 of the nucleotide sequence shown in SEQ ID NO: 31 or a probe that can be prepared from the nucleotide sequence under a stringent condition, showing homology of 60% or more, preferably 70% or more, more preferably 80% or more, to the foregoing nucleotide sequence, and having maltooligosyltrehalose synthase activity from a DNA coding for maltooligosyltrehalose synthase having a mutation or from a cell harboring it.

[0024] The "stringent condition" referred to herein is a condition under which so-called specific hybrid is formed, and non-specific hybrid is not formed. It is difficult to clearly express this condition by using any numerical value. However, for example, the stringent condition includes a condition under which DNA's having high homology, for example, DNA's having homology of not less than 55%, preferably not less than 60%, are hybridized with each other, and DNA's having homology lower than the above level are not hybridized with each other. Alternatively, the stringent condition is exemplified by a condition under which DNA's are hybridized with each other at a salt concentration corresponding to an ordinary condition of washing in Southern hybridization, i.e., 1 x SSC, 0.1 % SDS, preferably 0.1 x SSC, 0.1 % SDS, at 60°C.

[0025] As the probe, a partial sequence of each gene can also be used. Such a probe can be produced by PCR using oligonucleotides produced based on the nucleotide sequence of each gene as primers and a DNA fragment containing each gene as a template. When a DNA fragment in a length of about 300 bp is used as the probe, the washing conditions for the hybridization may consists of 50°C, 2 x SSC and 0.1% SDS.

[0026] Genes hybridizable under such conditions as described above include those having a stop codon generated in a coding region of the genes, and those having no activity due to mutation of active center. However, such mutants can be easily removed by ligating each of the genes with a commercially available expression vector, and measuring trehalose-6-phosphate synthase activity or maltooligosyltrehalose synthase activity.

[0027] When an *otsA* gene or *treY* gene is used for the disruption of these genes on chromosomes of coryneform bacteria, the encoded trehalose-6-phosphate synthase or maltooligosyltrehalose synthase are not required to have their activities. Further, the *otsA* gene or *treY* gene used for the gene disruption may be a gene derived from another microorganism, so long as they can undergo homologous recombination with these genes of coryneform bacteria. For example, an *otsA* gene of bacterium belonging to the genus *Escherichia* or *Mycobacterium, treY* gene of bacterium belonging to the genus *Arthrobacter, Brevibacterium helvolum*, or bacterium belonging to the genus *Rhizobium* can

EPODOC: <EP 1174508A2 >

be mentioned.

[0028] A deletion type gene of the *otsA* gene or *treY* gene can be prepared by excising a certain region with restriction enzyme(s) from a DNA fragment containing one of these genes or a part of them to delete at least a part of coding region or an expression regulatory sequence such as promoter.

[0029] Further, a deletion type gene can also be obtained by performing PCR using primers designed so that a part of gene should be deleted. Furthermore, a deletion type gene may be one obtained by single nucleotide mutation, for example, a frame shift mutation.

[0030] Gene disruption of the *otsA* gene will be explained hereafter. Gene disruption of the *treY* gene can be performed similarly.

[0031] An otsA gene on a host chromosome can be replaced with a deletion type *otsA* gene as follows. That is, a deletion type *otsA* gene and a marker gene for resistance to a drug, such as kanamycin, chloramphenicol, tetracycline and streptomycin, are inserted into a plasmid that cannot autonomously replicate in coryneform bacteria to prepare a recombinant DNA. A coryneform bacterium can be transformed with the recombinant DNA, and the transformant strain can be cultured in a medium containing the drug to obtain a transformant strain in which the recombinant DNA was introduced into chromosomal DNA. Alternatively, such a transformant strain can be obtained by using a temperature sensitive plasmid as the plasmid, and culturing the transformants at a temperature at which the temperature sensitive plasmid cannot replicate.

[0032] In a strain in which the recombinant DNA is incorporated into a chromosome as described above, the recombinant DNA causes recombination with an *otsA* gene sequence that originally exists on the chromosome, and two of fused genes comprising the chromosomal *otsA* gene and the deletion type *otsA* gene are inserted into the chromosome so that other portions of the recombinant DNA (vector portion and drug resistance marker gene) should be interposed between them.

[0033] Then, in order to leave only the deletion type *otsA* gene on the chromosomal DNA, one copy of the *otsA* gene is eliminated from the chromosomal DNA together with the vector portion (including the drug resistance marker gene) by recombination of two of the *otsA* genes. In that case, the normal otsA gene is left on the chromosomal DNA and the deletion type *otsA* gene is excised, or conversely, the deletion type *otsA* gene is left on the chromosomal DNA and the normal *otsA* gene is excised. It can be confirmed which type of the gene is left on the chromosomal DNA by investigating structure of the *otsA* gene on the chromosome by PCR, hybridization or the like.

[0034] The coryneform bacterium used for the present invention may have enhanced activity of an enzyme that catalyzes the biosynthesis of L-glutamic acid in addition to the deletion or decrease of trehalose synthesis ability. Examples of the enzyme that catalyzes the biosynthesis of L-glutamic acid include glutamate dehydrogenase, glutamine synthetase, glutamate synthase, isocitrate dehydrogenase, aconitate hydratase, citrate synthase, pyruvate carboxylase, phosphoenolpyruvate carboxylase, phosphoenolpyruvate synthase, enolase, phosphoglyceromutase, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, triosephosphate isomerase, fructose bisphosphate aldolase, phosphofructokinase, glucose phosphate isomerase and so forth.

[0035] Further, in the coryneform bacterium used for the present invention, an enzyme that catalyzes a reaction for generating a compound other than L-glutamic acid by branching off from the biosynthetic pathway of L-glutamic acid may be declined or made deficient. Examples of such an enzyme include α-ketoglutarate dehydrogenase, isocitrate lyase, phosphate acetyltransferase, acetate kinase, acetohydroximate synthase, acetolactate synthase, formate acetyltransferase, lactate dehydrogenase, L-glutamate decarboxylase, 1-pyrroline dehydrogenase and so forth.

[0036] Furthermore, by introducing a temperature sensitive mutation for a biotin activity inhibiting substance such as surface active agents into a coryneform bacterium having L-glutamic acid producing ability, the bacterium becomes to be able to produce L-glutamic acid in a medium containing an excessive amount of biotin in the absence of a biotin activity inhibiting substance (see WO96/06180). As such a coryneform bacterium, the *Brevibacterium lactofermentum* AJ13029 strain disclosed in WO96/06180 can be mentioned. The AJ13029 strain was deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (currently, the independent administrative corporation, National Institute of Advanced Industrial Science and Technology, International Patent Organism Depositary (Chuo Dai-6, 1-1 Higashi 1-Chome, Tsukuba-shi, Ibaraki-ken, Japan, postal code: 305-5466) on September 2, 1994, and received an accession number of FERM P-14501. Then, it was transferred to an international deposit under the provisions of the Budapest Treaty on August 1, 1995, and received an accession number of FERM BP-5189.

[0037] When a coryneform bacterium having L-glutamic acid producing ability, in which trehalose synthesis ability is decreased or deleted, is cultured in a suitable medium, L-glutamic acid is accumulated in the medium.

[0038] The medium used for producing L-glutamic acid is a usual medium that contains a carbon source, a nitrogen source, inorganic ions and other organic trace nutrients as required. As the carbon source, it is possible to use sugars such as glucose, lactose, galactose, fructose, sucrose, maltose, blackstrap molasses and starch hydrolysate; alcohols such as ethanol and inositol; or organic acids such as acetic acid, fumaric acid, citric acid and succinic acid.

[0039] As the nitrogen source, there can be used inorganic ammonium salts such as ammonium sulfate, ammonium

nitrate, ammonium chloride, ammonium phosphate and ammonium acetate, ammonia, organic nitrogen such as peptone, meat extract, yeast extract, corn steep liquor and soybean hydrolysate, ammonia gas, aqueous ammonia and so forth.

[0040] As the inorganic ions (or sources thereof), added is a small amount of potassium phosphate, magnesium sulfate, iron ions, manganese ions and so forth. As for the organic trace nutrients, it is desirable to add required substances such as vitamin B_1, yeast extract and so forth in a suitable amount as required.

[0041] The culture is preferably performed under an aerobic condition performed by shaking, stirring for aeration or the like for 16 to 72 hours. The culture temperature is controlled to be at 30°C to 45°C, and pH is controlled to be 5 to 9 during the culture. For such adjustment of pH, inorganic or organic acidic or alkaline substances, ammonia gas and so forth can be used.

[0042] Collection of L-glutamic acid from fermentation broth can be performed by, for example, methods utilizing ion exchange resins, crystallization and so forth. Specifically, L-glutamic acid can be adsorbed on an anion exchange resin and isolated from it, or crystallized by neutralization.

EXAMPLES

[0043] Hereafter, the present invention will be explained more specifically with reference to the following examples.

Example 1: Construction of *otsA* gene-disrupted strain of *Brevibacterium lactoferinentum*

<1> Cloning of *otsA* gene

[0044] Since *otsA* gene of *Brevibacterium lactofermentum* was not known, it was obtained by utilizing a nucleotide sequence of *otsA* gene of another microorganism for reference. The *otsA* genes of *Escherichia* and *Mycobacterium* had been hitherto elucidated for their entire nucleotide sequences (Kaasen I., *et al., Gene, 145* (1), 9-15 (1994); De Smet K.A., *et al., Microbiology, 146* (1), 199-208 (2000)). Therefore, referring to an amino acid sequence deduced from these nucleotide sequences, DNA primers P1 (SEQ ID NO: 1) and P2 (SEQ ID NO: 2) for PCR were synthesized first. The DNA primers P1 and P2 corresponded to the regions of the nucleotide numbers of 1894-1913 and 2531-2549 of the nucleotide sequence of the otsA gene of *Escherichia coli* (GenBank accession X69160), respectively. They also corresponded to the regions of the nucleotide numbers 40499-40518 and 41166-41184 of the *otsA* gene of *Mycobacterium tuberculosis* (GenBank accession Z95390), respectively.

[0045] Then, PCR was performed by using the primers P1 and P2 and chromosomal DNA of *Brevibacterium lactofermentum* ATCC 13869 as a template with a cycle consisting of reactions at 94°C for 0.5 minute, 50°C for 0.5 minute and 72°C for 4 minutes, which was repeated for 30 cycles. As a result, a substantially single kind of amplified fragment of about 0.6 kbp was obtained. This amplified fragment was cloned into a plasmid vector pCR2.1 by using "Original TA Cloning Kit" produced by Invitrogen to obtain pCotsA. Then, the nucleotide sequence of the cloned fragment was determined.

[0046] Based on the nucleotide sequence of the partial fragment of *otsA* gene obtained as described above, DNA primers P10 (SEQ ID NO: 8) and P12 (SEQ ID NO: 10) were newly synthesized, and unknown regions flanking to the partial fragment was amplified by "inverse PCR" (Triglia, T. *et al., Nucleic Acids Res., 16,* 81-86 (1988); Ochman H., *et al., Genetics, 120,* 621-623 (1988)). The chromosomal DNA of *Brevibacterium lactofermentum* ATCC 13869 was digested with a restriction enzyme *Bam*HI, *Bgl*II, *Cla*I, *Hind*III, *Kpn*I, *Mlu*I, *Mun*I, *Sal*I or *Xho*I, and self-ligated by using T4 DNA ligase (Takara Shuzo). By using resultant DNA as a template and the DNA primers P10 and P12, PCR was performed with a cycle consisting of reactions at 94°C for 0.5 minute, 55°C for 1 minute and 72°C for 4 minutes, which was repeated for 30 cycles. As a result, when *Cla*I or *Bgl*II was used as the restriction enzyme, an amplified fragment of 4 kbp was obtained for each case. The nucleotide sequences of these amplified fragments were directly determined by using the DNA primers P5 to P9 (SEQ ID NOS: 3-7) and P11 to P15 (SEQ ID NOS: 9-13). Thus, the entire nucleotide sequence of *otsA* gene of *Brevibacterium lactofermentum* ATCC 13869 was determined as shown in SEQ ID NO: 29. The amino acid sequence encoded by this nucleotide sequence is shown in SEQ ID NOS: 29 and 30.

[0047] When homology of the sequence of the aforementioned *otsA* gene was determined with respect to the *otsA* gene of *Escherichia coli* (GenBank accession X69160) and *the otsA* gene of *Mycobacterium tuberculosis* (GenBank accession Z95390), the nucleotide sequence showed homologies of 46.3% and 55.9%, respectively, and the amino acid sequence showed homologies of 30.9% and 51.7%, respectively. The homologies were calculated by using software, "GENETIX-WIN" (Software Development), based on the Lipman-Person method *(Science, 227,* 1435-1441 (1985)).

<2> Preparation of plasmid for *otsA* gene disruption

[0048] In order to examine presence or absence of improvement effect in L-glutamic acid productivity by disruption of a gene coding for an enzyme in trehalose biosysthesis pathway in coryneform bacteria, a plasmid for *otsA* gene disruption was produced. A plasmid for *otsA* gene disruption was produced as follows. PCR was performed by using the plasmid pCotsA previously constructed in the cloning of the *otsA* gene as a template and the primers P29 (SEQ ID NO: 33) and P30 (SEQ ID NO: 34) comprising *Cla*I site with a cycle consisting of reactions at 94°C for 0.5 minute, 55°C for 0.5 minute and 72°C for 8 minutes, which was repeated for 30 cycles. The amplified fragment was digested with *Cla*I, blunt-ended by using T4 DNA polymerase (Takara Shuzo), and self-ligated by using T4 ligase (Takara Shuzo) to construct a plasmid pCotsAC containing the otsA gene having a frame shift mutation (1258-1300th nucleotides of SEQ ID NO: 29 were deleted) at an approximately central part thereof.

<3> Preparation of *otsA* gene-disrupted strain

[0049] By using the plasmid pCotsAC for gene disruption, a L-glutamic acid producing bacterium, *Brevibacterium lactofermentum* ATCC 13869, was transformed by the electric pulse method, and transformants were selected as to the ability to grow in CM2B medium containing 20 mg/L of kanamycin. Because the plasmid pCotsAC for *otsA* gene disruption did not have a replication origin that could function in *Brevibacterium lactofermentum*, resultant transformants obtained by using the plasmid suffered homologous recombination occurred between the *otsA* genes on the chromosome of *Brevibacterium lactofermentum* and the plasmid pCotsAC for gene disruption. From the homologous recombinant strains obtained as described above, strains in which the vector portion of the plasmid pCotsAC for gene disruption was eliminated due to re-occurrence of homologous recombination were selected based on acquired kanamycin sensitivity as a marker.

[0050] From the strains obtained as described above, a strain introduced with the desired frame shift mutation was selected. Selection of such a strain was performed by PCR using chromosomal DNA extracted from a strain that became kanamycin sensitive as a template and the DNA primers P8 (SEQ ID NO: 14) and P13 (SEQ ID NO: 11) with a cycle consisting of reactions at 94°C for 0.5 minute, 55°C for 0.5 minute and 72°C for 1 minutes, which was repeated for 30 cycles, and sequencing of the obtained amplified fragment using the DNA primer P8 to confirm disfunction of the *otsA* gene due to introduction of frame shift mutation. The strain obtained as described above was designated as AOA strain.

Example 2: Construction of *trey* gene-disrupted strain

<1> Cloning of *treY* gene

[0051] Since *treY* gene of *Brevibacterium lactofermentum* was not known, it was obtained by using nucleotide sequences of *treY* genes of the other microorganisms for reference. The nucleotide sequences of *treY* genes were hitherto elucidated for the genera Arthrobacter, *Brevibacterium* and *Rhizobium* (Maruta K., *et al.*, *Biochim. Biophys. Acta, 1289* (1), 10-13 (1996); Genbank accession AF039919; Maruta K., *et al.*, *Biosci. Biotechnol. Biochem.*, *60* (4), 717-720 (1996)). Therefore, referring to an amino acid sequence deduced from these nucleotide sequences, the PCR DNA primers P3 (SEQ ID NO: 14) and P4 (SEQ ID NO: 15) were synthesized first. The DNA primers P3 and P4 correspond to the regions of the nucleotide numbers of 975-992 and 2565-2584 of the nucleotide sequence of the *treY* gene of *Arthrobacter* species (GenBank accession D63343), respectively. Further, they correspond to the regions of the nucleotide numbers 893-910 and 2486-2505 of the *treY* gene of *Brevibacterium helvolum* (GenBank accession AF039919), respectively. Furthermore, they correspond to the regions of the nucleotide numbers of 862-879 and 2452-2471 of *treY* gene of *Rhizobium* species (GenBank accession D78001).

[0052] Then, PCR was performed by using the primers P3 and P4 and chromosomal DNA of *Brevibacterium lactofermentum* ATCC13869 as a template with a cycle consisting of reactions at 94°C for 0.5 minute, 55°C for 0.5 minute and 72°C for 2 minutes, which was repeated for 30 cycles. As a result, a substantially single kind of an amplified fragment of about 1.6 kbp was obtained. This amplified fragment was cloned into a plasmid vector pCR2.1 by using "Original TA Cloning Kit" produced by Invitrogen. Then, the nucleotide sequence was determined for about 0.6 kb.

[0053] Based on the nucleotide sequence of the partial fragment of *treY* gene obtained as described above, the DNA primers P16 (SEQ ID NO: 16) and P26 (SEQ ID NO: 26) were newly synthesized, and unknown regions flanking to the partial fragment was amplified by "inverse PCR" (Triglia, T. *et al., Nucleic Acids Res., 16,* 81-86 (1988); Ochman H., *et al., Genetics, 120,* 621-623 (1988)). The chromosomal DNA of *Brevibacterium lactofermentum* ATCC 13869 was digested with a restriction enzyme *Bam*HI, *Hin*dIII, *Sal*I or *Xho*I, and self-ligated by using T4 DNA ligase (Takara Shuzo). By using this as a template and the DNA primers P16 and P26, PCR was performed with a cycle consisting of reactions at 94°C for 0.5 minute, 55°C for 1 minute and 72°C for 4 minutes, which was repeated for 30 cycles. As a result, when

*Hind*III or *Sal*I was used as the restriction enzyme, an amplified fragment of 0.6 kbp or 1.5 kbp was obtained, respectively. The nucleotide sequences of these amplified fragments were directly determined by using the DNA primers P16 to P28 (SEQ ID NOS: 16-28). Thus, the entire nucleotide sequence of *treY* gene of *Brevibacterium lactofermentum* ATCC 13869 was determined as shown in SEQ ID NO: 31. The amino acid sequence encoded by this nucleotide sequence is shown in SEQ ID NOS: 31 and 32.

[0054] When homology of the sequence of the aforementioned *treY* gene was determined with respect to the *treY* gene of *Arthrobacter* sp. (GenBank accession D63343), *treY* gene of *Brevibacterium helvolum* (GenBank accession AF039919) and *treY* gene of *Rhizobium* sp. (GenBank accession D78001), the nucleotide sequence showed homologies of 52.0%, 52.3% and 51.9%, respectively, and the amino acid sequence showed homologies of 40.9%, 38.5% and 39.8%, respectively. The homologies were calculated by using software, "GENETIX-WIN" (Software Development), based on the Lipman-Person method *(Science, 227,* 1435-1441 (1985)).

<2> Preparation of plasmid for *treY* gene disruption

[0055] In order to examine presence or absence of improvement effect in L-glutamic acid productivity by disruption of the gene coding for the enzyme in trehalose biosysthesis pathway in coryneform bacteria, a plasmid for *treY* gene disruption was produced. First, PCR was performed by using the primers P17 (SEQ ID NO: 17) and P25 (SEQ ID NO: 25) and the chromosomal DNA of ATCC 13869 as a template with a cycle consisting of reactions at 94°C for 0.5 minute, 60°C for 0.5 minute and 72°C for 2 minutes, which was repeated for 30 cycles. The amplified fragment was digested with *Eco*RI and ligated to pHSG299 (Takara Shuzo) digested with *Eco*RI by using T4 DNA ligase (Takara Shuzo) to obtain a plasmid pHtreY. Further, this pHtreY was digested with *Aff*II (Takara Shuzo), blunt-ended by using T4 DNA polymerase (Takara Shuzo), and self-ligated by using T4 ligase (Takara Shuzo) to construct a plasmid pHtreYA containing the *treY* gene having a frame shift mutation (four nucleotides were inserted after the 1145th nucleotide in the sequence of SEQ ID NO: 31) at an approximately central part thereof.

<3> Preparation of *treY* gene-disrupted strain

[0056] By using the plasmid pCtreYA for gene disruption, a L-glutamic acid producing bacterium, *Brevibacterium lactofermentum* ATCC 13869, was transformed by the electric pulse method, and transformants were selected as to the ability to grow in CM2B medium containing 20 mg/L of kanamycin. Because the plasmid pCtreYA for *treY* gene disruption does not have a replication origin that could function in *Brevibacterium lactofermentum,* the transformants obtained by using the plasmid suffered recombination occurred between the *treY* genes on the *Brevibacterium lactofermentum* chromosome and the plasmid pCtreYA for gene disruption. From the homologous recombinant strains obtained as described above, strains in which the vector portion of the plasmid pCtreYA for gene disruption was eliminated due to re-occurrence of homologous recombination were selected based on acquired kanamycin sensitivity as a marker.

[0057] From the strains obtained as described above, a strain introduced with the desired frame shift mutation was selected. Selection of such a strain was performed by PCR using the DNA primers P19 (SEQ ID NO: 19) and P25 (SEQ ID NO: 25) with a cycle consisting of reactions at 94°C for 0.5 minute, 55°C for 0.5 minute and 72°C for 1.5 minutes, which was repeated for 30 cycles, and sequencing the obtained fragment using the DNA primer P21 or P23 to confirm dysfunction of the *treY* gene due to introduction of frame shift mutation. The strain obtained as described above was designated as ΔTA strain.

Example 3: Evaluation of L-glutamic acid producing ability of ΔOA strain and ΔTA strain

[0058] The ATCC 13869 strain, ΔOA strain and ΔTA strain were each cultured for producing L-glutamic acid as follows. Each of these strains was refreshed by culturing it on a CM2B plate medium, and each refreshed strain was cultured in a medium containing 80 g of glucose, 1 g of KH_2PO_4, 0.4 g of $MgSO_4$, 30 g of $(NH_4)_2SO_4$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 0.01 g $MnSO_4 \cdot 7H_2O$, 15 ml of soybean hydrolysate solution, 200 μg of thiamin hydrochloride, 3 μg of biotin and 50 g of $CaCO_3$ in 1 L of pure water (adjusted to pH 8.0 with KOH) at 31.5°C. After the culture, amount of L-glutamic acid accumulated in the medium and absorbance at 620 nm of the culture broth diluted 51 times were measured. The results are shown in Table 1.

[0059] The *Brevibacterium lactofermentum* strains of which *otsA* gene or *treY* gene was disrupted showed growth in a degree similar to that of the parent strain, and in addition, increased L-glutamic acid production compared with the parent strain.

BNSDOCID: <EP 1174508A2 I >

Table 1

Strain	OD_{620} (x51)	L-Glutamic acid (g/L)	Yield (%)
ATCC 13869	0.930	40.2	48.4
ΔOA	1.063	43.8	52.8
ΔTA	0.850	45.6	54.9

(Explanation of sequence Listing)

[0060]

SEQ ID NO: 1: Primer P1 for amplification of *otsA*
SEQ ID NO: 2: Primer P2 for amplification of *otsA*
SEQ ID NO: 3: Primer P5
SEQ ID NO: 4: Primer P6
SEQ ID NO: 5: Primer P7
SEQ ID NO: 6: Primer P8
SEQ ID NO: 7: Primer P9
SEQ ID NO: 8: Primer P10
SEQ ID NO: 9: Primer P11
SEQ ID NO: 10: Primer P12
SEQ ID NO: 11: Primer P13
SEQ ID NO: 12: Primer P14
SEQ ID NO: 13: Primer P15
SEQ ID NO: 14: Primer P3 for amplification of *treY*
SEQ ID NO: 15: Primer P4 for amplification of *treY*
SEQ ID NO: 16: Primer P16
SEQ ID NO: 17: Primer P17
SEQ ID NO: 18: Primer P18
SEQ ID NO: 19: Primer P19
SEQ ID NO: 20: Primer P20
SEQ ID NO: 21: Primer P21
SEQ ID NO: 22: Primer P22
SEQ ID NO: 23: Primer P23
SEQ ID NO: 24: Primer P24
SEQ ID NO: 25: Primer P25
SEQ ID NO: 26: Primer P26
SEQ ID NO: 27: Primer P27
SEQ ID NO: 28: Primer P28
SEQ ID NO: 29: Nucleotide sequence of *otsA* gene
SEQ ID NO: 30: Amino acid sequence of OtsA
SEQ ID NO: 31: Nucleotide sequence of *treY* gene
SEQ ID NO: 32: Amino acid sequence of TreY
SEQ ID NO: 33: Primer P29
SEQ ID NO: 34: Primer P30

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EPODOC: <EP 1174508A2 >

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245 250 255
Asp Val Glu Met Phe Gly Glu Ala Ser Lys Ser Ala Val Leu Asp Leu
260 265 270
Leu Lys Thr Leu Asp Glu Pro Glu Thr Val Phe Leu Gly Val Asp Arg
275 280 285
Leu Asp Tyr Thr Lys Gly Ile Leu Gln Arg Leu Leu Ala Phe Glu Glu
290 295 300
Leu Leu Glu Ser Gly Ala Leu Glu Ala Asp Lys Ala Val Leu Leu Gln
305 310 315 320
Val Ala Thr Pro Ser Arg Glu Arg Ile Asp His Tyr Arg Val Ser Arg
325 330 335
Ser Gln Val Glu Glu Ala Val Gly Arg Ile Asn Gly Arg Phe Gly Arg
340 345 350
Met Gly Arg Pro Val Val His Tyr Leu His Arg Ser Leu Ser Lys Asn
355 360 365
Asp Leu Gln Val Leu Tyr Thr Ala Ala Asp Val Met Leu Val Thr Pro
370 375 380
Phe Lys Asp Gly Met Asn Leu Val Ala Lys Glu Phe Val Ala Asn His
385 390 395 400
Arg Asp Gly Thr Gly Ala Leu Val Leu Ser Glu Phe Ala Gly Ala Ala
405 410 415
Thr Glu Leu Thr Gly Ala Tyr Leu Cys Asn Pro Phe Asp Val Glu Ser
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Ile Lys Arg Gln Met Val Ala Ala Val His Asp Leu Lys His Asn Pro
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Glu Ser Ala Ala Thr Arg Met Lys Thr Asn Ser Glu Gln Val Tyr Thr
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His Asp Val Asn Val Trp Ala Asn Ser Phe Leu Asp Cys Leu Ala Gln

BNSDOCID: <EP 1174508A2 I >

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Ser Gly Glu Asn Ser
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aacttttaag ggtgtttcat c atg gca cgt cca att tcc gca acg tac agg   111
                       Met Ala Arg Pro Ile Ser Ala Thr Tyr Arg
                         1               5                  10
ctt caa atg cga gga cct caa gca gat agc gcc ggg cgt ttc ttt ggt   159
Leu Gln Met Arg Gly Pro Gln Ala Asp Ser Ala Gly Arg Phe Phe Gly
                 15                  20                  25
ttt gcg cag gcc aaa gcc cag ctt ccc tat ctg aag aag cta ggc atc   207
Phe Ala Gln Ala Lys Ala Gln Leu Pro Tyr Leu Lys Lys Leu Gly Ile
             30                  35                  40
agc cac ctg tac ctc tcc cct att ttt acg gcc atg cca gat tcc aat   255
Ser His Leu Tyr Leu Ser Pro Ile Phe Thr Ala Met Pro Asp Ser Asn
         45                  50                  55
cat ggc tac gat gtc att gat ccc acc gcc atc aat gaa gag ctc ggt   303
His Gly Tyr Asp Val Ile Asp Pro Thr Ala Ile Asn Glu Glu Leu Gly
     60                  65                  70
ggc atg gag ggt ctt cga gat ctt gct gca gct aca cac gag ttg ggc   351
Gly Met Glu Gly Leu Arg Asp Leu Ala Ala Ala Thr His Glu Leu Gly
 75                  80                  85                  90
atg ggc atc atc att gat att gtt ccc aac cat tta ggt gtt gcc gtt   399
Met Gly Ile Ile Ile Asp Ile Val Pro Asn His Leu Gly Val Ala Val
                 95                 100                 105
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cca cat ttg aat cct tgg tgg tgg gat gtt cta aaa aac ggc aaa gat   447
Pro His Leu Asn Pro Trp Trp Trp Asp Val Leu Lys Asn Gly Lys Asp
            110                 115                 120
tcc gct ttt gag ttc tat ttc gat att gac tgg cac gaa gac aac ggt   495
Ser Ala Phe Glu Phe Tyr Phe Asp Ile Asp Trp His Glu Asp Asn Gly
        125                 130                 135
tct ggt ggc aag ctg ggc atg ccg att ctg ggt gct gaa ggc gat gaa   543
Ser Gly Gly Lys Leu Gly Met Pro Ile Leu Gly Ala Glu Gly Asp Glu
    140                 145                 150
gac aag ctg gaa ttc gcg gag ctt gat gga gag aaa gtg ctc aaa tat   591
Asp Lys Leu Glu Phe Ala Glu Leu Asp Gly Glu Lys Val Leu Lys Tyr
155                 160                 165                 170
ttt gac cac ctc ttc cca atc gcg cct ggt acc gaa gaa ggg aca ccg   639
Phe Asp His Leu Phe Pro Ile Ala Pro Gly Thr Glu Glu Gly Thr Pro
                175                 180                 185
caa gaa gtc tac aag cgc cag cat tac cgc ctg cag ttc tgg cgc gac   687
Gln Glu Val Tyr Lys Arg Gln His Tyr Arg Leu Gln Phe Trp Arg Asp
            190                 195                 200
ggc gtg atc aac ttc cgt cgc ttc ttt tcc gtg aat acg ttg gct ggc   735
Gly Val Ile Asn Phe Arg Arg Phe Phe Ser Val Asn Thr Leu Ala Gly
        205                 210                 215
atc agg caa gaa gat ccc ttg gtg ttt gaa cat act cat cgt ctg ctg   783
Ile Arg Gln Glu Asp Pro Leu Val Phe Glu His Thr His Arg Leu Leu
    220                 225                 230
cgc gaa ttg gtg gcg gaa gac ctc att gac ggc gtg cgc gtc gat cac   831
Arg Glu Leu Val Ala Glu Asp Leu Ile Asp Gly Val Arg Val Asp His
235                 240                 245                 250
ccc gac ggg ctt tcc gat cct ttt gga tat ctg cac aga ctc cgc gac   879
Pro Asp Gly Leu Ser Asp Pro Phe Gly Tyr Leu His Arg Leu Arg Asp
                255                 260                 265
ctc att gga cct gac cgc tgg ctg atc atc gaa aag atc ttg agc gtt   927
Leu Ile Gly Pro Asp Arg Trp Leu Ile Ile Glu Lys Ile Leu Ser Val
            270                 275                 280
gat gaa cca ctc gat ccc cgc ctg gcc gtt gat ggc acc act ggc tac   975
Asp Glu Pro Leu Asp Pro Arg Leu Ala Val Asp Gly Thr Thr Gly Tyr
        285                 290                 295
gac ccc ctc cgt gaa ctc gac ggc gtg ttt atc tcc cga gaa tct gag   1023
Asp Pro Leu Arg Glu Leu Asp Gly Val Phe Ile Ser Arg Glu Ser Glu
    300                 305                 310
gac aaa ttc tcc atg ttg gcg ctg acc cac agt gga tcc acc tgg gat   1071
Asp Lys Phe Ser Met Leu Ala Leu Thr His Ser Gly Ser Thr Trp Asp
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gaa cgc gcc cta aaa tcc acg gag gaa agc ctc aaa cga gtc gtc gcg   1119
Glu Arg Ala Leu Lys Ser Thr Glu Glu Ser Leu Lys Arg Val Val Ala
                335                 340                 345
caa caa gaa ctc gca gcc gaa atc tta agg ctc gcc cgc gcc atg cgc   1167
Gln Gln Glu Leu Ala Ala Glu Ile Leu Arg Leu Ala Arg Ala Met Arg
            350                 355                 360
cgc gat aac ttc tcc acc gca ggc acc aac gtc acc gaa gac aaa ctt   1215
Arg Asp Asn Phe Ser Thr Ala Gly Thr Asn Val Thr Glu Asp Lys Leu
        365                 370                 375
agc gaa acc atc atc gaa tta gtc gcc gcc atg ccc gtc tac cgc gcc   1263
Ser Glu Thr Ile Ile Glu Leu Val Ala Ala Met Pro Val Tyr Arg Ala
    380                 385                 390
gac tac atc tcc ctc tca cgc acc acc gcc acc gtc atc gcg gag atg   1311
Asp Tyr Ile Ser Leu Ser Arg Thr Thr Ala Thr Val Ile Ala Glu Met
395                 400                 405                 410
tcc aaa cgc ttc ccc tcc cgg cgc gac gca ctc gac ctc atc tcg gcc   1359
Ser Lys Arg Phe Pro Ser Arg Arg Asp Ala Leu Asp Leu Ile Ser Ala
                415                 420                 425
gcc cta ctt ggc aat ggc gag gcc aaa atc cgc ttc gcc caa gtc tgc   1407
Ala Leu Leu Gly Asn Gly Glu Ala Lys Ile Arg Phe Ala Gln Val Cys
            430                 435                 440
ggc gcc gtc atg gcc aaa ggt gtg gaa gac acc acc ttc tac cgc gca   1455
Gly Ala Val Met Ala Lys Gly Val Glu Asp Thr Thr Phe Tyr Arg Ala
        445                 450                 455
tct agg ctc gtt gca ctg caa gaa gtc ggt ggc gcg ccg ggc agg ttc   1503
Ser Arg Leu Val Ala Leu Gln Glu Val Gly Gly Ala Pro Gly Arg Phe
    460                 465                 470
ggc gtc tcc gct gca gaa ttc cac ttg ctg cag gaa gaa cgc agc ctg   1551
Gly Val Ser Ala Ala Glu Phe His Leu Leu Gln Glu Glu Arg Ser Leu
475                 480                 485                 490
ctg tgg cca cgc acc atg acc acc ttg tcc acg cac gac acc aaa cgc   1599
Leu Trp Pro Arg Thr Met Thr Thr Leu Ser Thr His Asp Thr Lys Arg
                495                 500                 505
ggc gaa gat acc cgc gcc cgc atc atc tcc ctg tcc gaa gtc ccc gat   1647
Gly Glu Asp Thr Arg Ala Arg Ile Ile Ser Leu Ser Glu Val Pro Asp
            510                 515                 520
atg tac tcc gag ctg gtc aat cgt gtt ttc gca gtg ctc ccc gcg cca   1695
Met Tyr Ser Glu Leu Val Asn Arg Val Phe Ala Val Leu Pro Ala Pro
        525                 530                 535
gac ggc gca acg ggc agt ttc ctc cta caa aac ctg ctg ggc gta tgg   1743
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Asp Gly Ala Thr Gly Ser Phe Leu Leu Gln Asn Leu Leu Gly Val Trp
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ccc gcc gac ggc gtg atc acc gat gcg ctg cgc gat cga ttc agg gaa   1791
Pro Ala Asp Gly Val Ile Thr Asp Ala Leu Arg Asp Arg Phe Arg Glu
555                 560                 565                 570
tac gcc cta aaa gct atc cgc gaa gca tcc aca aaa acc acg tgg gtg   1839
Tyr Ala Leu Lys Ala Ile Arg Glu Ala Ser Thr Lys Thr Thr Trp Val
                575                 580                 585
gac ccc aac gag tcc ttc gag gct gcg gtc tgc gat tgg gtg gaa gcg   1887
Asp Pro Asn Glu Ser Phe Glu Ala Ala Val Cys Asp Trp Val Glu Ala
            590                 595                 600
ctt ttc gac gga ccc tcc acc tca tta atc acc gaa ttt gtc tcc cac   1935
Leu Phe Asp Gly Pro Ser Thr Ser Leu Ile Thr Glu Phe Val Ser His
        605                 610                 615
atc aac cgt ggc tct gtg aat atc tcc tta ggt agg aaa ctg ctg caa   1983
Ile Asn Arg Gly Ser Val Asn Ile Ser Leu Gly Arg Lys Leu Leu Gln
    620                 625                 630
atg gtg ggc gct gga atc ccc gac act tac caa gga act gag ttt tta   2031
Met Val Gly Ala Gly Ile Pro Asp Thr Tyr Gln Gly Thr Glu Phe Leu
635                 640                 645                 650
gaa gac tcc ctg gta gat ccc gat aac cga cgc ttt gtt gat tac acc   2079
Glu Asp Ser Leu Val Asp Pro Asp Asn Arg Arg Phe Val Asp Tyr Thr
                655                 660                 665
gcc aga gaa caa gtc ctg gag cgc ctg caa acc tgg gat tgg acg cag   2127
Ala Arg Glu Gln Val Leu Glu Arg Leu Gln Thr Trp Asp Trp Thr Gln
            670                 675                 680
gtt aat tcg gta gaa gac ttg gtg gat aac gcc gac atc gcc aaa atg   2175
Val Asn Ser Val Glu Asp Leu Val Asp Asn Ala Asp Ile Ala Lys Met
        685                 690                 695
gcc gtg gtc cat aaa tcc ctc gag ttg cgt gct gaa ttt cgt gca agc   2223
Ala Val Val His Lys Ser Leu Glu Leu Arg Ala Glu Phe Arg Ala Ser
    700                 705                 710
ttt gtt ggt gga gat cat cag gca gta ttt ggc gaa ggt cgc gca gaa   2271
Phe Val Gly Gly Asp His Gln Ala Val Phe Gly Glu Gly Arg Ala Glu
715                 720                 725                 730
tcc cac atc atg ggc atc gcc cgc ggt aca gac cga aac cac ctc aac   2319
Ser His Ile Met Gly Ile Ala Arg Gly Thr Asp Arg Asn His Leu Asn
                735                 740                 745
atc att gct ctt gct acc cgt cga cca ctg atc ttg gaa gac cgt ggc   2367
Ile Ile Ala Leu Ala Thr Arg Arg Pro Leu Ile Leu Glu Asp Arg Gly
            750                 755                 760
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agg ctc acc ggg caa cgc ttc agt ggt gtt gtc cca gcc acc gat ttg 2463
Arg Leu Thr Gly Gln Arg Phe Ser Gly Val Val Pro Ala Thr Asp Leu
780 785 790
ttc tca cat tta ccc gta tct ttg ttg gtt tta gta ccc gat agt gag 2511
Phe Ser His Leu Pro Val Ser Leu Leu Val Leu Val Pro Asp Ser Glu
795 800 805 810
ttt tgatccctgc acaggaaagt tagcggcgct actatgaacg atcgatatgt 2564
Phe
ctgacaacac tctctcccaa tttggcagtt actaccacga attccgacgt gcccatccca 2624
tggccgacgt cgaattcctc ctagcaattg aagaattact cacagacggt ggtgtcacct 2684
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gtgttcggat caccacgtac cactccacgg aaattcccgt ggccttaaaa gtgctccaag 2864
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35 40 45
Pro Ile Phe Thr Ala Met Pro Asp Ser Asn His Gly Tyr Asp Val Ile
50 55 60
Asp Pro Thr Ala Ile Asn Glu Glu Leu Gly Gly Met Glu Gly Leu Arg
65 70 75 80
Asp Leu Ala Ala Ala Thr His Glu Leu Gly Met Gly Ile Ile Ile Asp
85 90 95
Ile Val Pro Asn His Leu Gly Val Ala Val Pro His Leu Asn Pro Trp
100 105 110
Trp Trp Asp Val Leu Lys Asn Gly Lys Asp Ser Ala Phe Glu Phe Tyr
115 120 125

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Phe Asp Ile Asp Trp His Glu Asp Asn Gly Ser Gly Gly Lys Leu Gly
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Met Pro Ile Leu Gly Ala Glu Gly Asp Glu Asp Lys Leu Glu Phe Ala
145                 150                 155                 160
Glu Leu Asp Gly Glu Lys Val Leu Lys Tyr Phe Asp His Leu Phe Pro
                165                 170                 175
Ile Ala Pro Gly Thr Glu Glu Gly Thr Pro Gln Glu Val Tyr Lys Arg
            180                 185                 190
Gln His Tyr Arg Leu Gln Phe Trp Arg Asp Gly Val Ile Asn Phe Arg
        195                 200                 205
Arg Phe Phe Ser Val Asn Thr Leu Ala Gly Ile Arg Gln Glu Asp Pro
    210                 215                 220
Leu Val Phe Glu His Thr His Arg Leu Leu Arg Glu Leu Val Ala Glu
225                 230                 235                 240
Asp Leu Ile Asp Gly Val Arg Val Asp His Pro Asp Gly Leu Ser Asp
                245                 250                 255
Pro Phe Gly Tyr Leu His Arg Leu Arg Asp Leu Ile Gly Pro Asp Arg
            260                 265                 270
Trp Leu Ile Ile Glu Lys Ile Leu Ser Val Asp Glu Pro Leu Asp Pro
        275                 280                 285
Arg Leu Ala Val Asp Gly Thr Thr Gly Tyr Asp Pro Leu Arg Glu Leu
    290                 295                 300
Asp Gly Val Phe Ile Ser Arg Glu Ser Glu Asp Lys Phe Ser Met Leu
305                 310                 315                 320
Ala Leu Thr His Ser Gly Ser Thr Trp Asp Glu Arg Ala Leu Lys Ser
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Thr Glu Glu Ser Leu Lys Arg Val Val Ala Gln Gln Glu Leu Ala Ala
            340                 345                 350
Glu Ile Leu Arg Leu Ala Arg Ala Met Arg Arg Asp Asn Phe Ser Thr
        355                 360                 365
Ala Gly Thr Asn Val Thr Glu Asp Lys Leu Ser Glu Thr Ile Ile Glu
    370                 375                 380
Leu Val Ala Ala Met Pro Val Tyr Arg Ala Asp Tyr Ile Ser Leu Ser
385                 390                 395                 400
Arg Thr Thr Ala Thr Val Ile Ala Glu Met Ser Lys Arg Phe Pro Ser
                405                 410                 415
Arg Arg Asp Ala Leu Asp Leu Ile Ser Ala Ala Leu Leu Gly Asn Gly
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Glu Ala Lys Ile Arg Phe Ala Gln Val Cys Gly Ala Val Met Ala Lys
        435                 440                 445
Gly Val Glu Asp Thr Thr Phe Tyr Arg Ala Ser Arg Leu Val Ala Leu
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Thr Thr Leu Ser Thr His Asp Thr Lys Arg Gly Glu Asp Thr Arg Ala
            500                 505                 510
Arg Ile Ile Ser Leu Ser Glu Val Pro Asp Met Tyr Ser Glu Leu Val
        515                 520                 525
Asn Arg Val Phe Ala Val Leu Pro Ala Pro Asp Gly Ala Thr Gly Ser
    530                 535                 540
Phe Leu Leu Gln Asn Leu Leu Gly Val Trp Pro Ala Asp Gly Val Ile
545                 550                 555                 560
Thr Asp Ala Leu Arg Asp Arg Phe Arg Glu Tyr Ala Leu Lys Ala Ile
                565                 570                 575
Arg Glu Ala Ser Thr Lys Thr Thr Trp Val Asp Pro Asn Glu Ser Phe
            580                 585                 590
Glu Ala Ala Val Cys Asp Trp Val Glu Ala Leu Phe Asp Gly Pro Ser
        595                 600                 605
Thr Ser Leu Ile Thr Glu Phe Val Ser His Ile Asn Arg Gly Ser Val
    610                 615                 620
Asn Ile Ser Leu Gly Arg Lys Leu Leu Gln Met Val Gly Ala Gly Ile
625                 630                 635                 640
Pro Asp Thr Tyr Gln Gly Thr Glu Phe Leu Glu Asp Ser Leu Val Asp
                645                 650                 655
Pro Asp Asn Arg Arg Phe Val Asp Tyr Thr Ala Arg Glu Gln Val Leu
            660                 665                 670
Glu Arg Leu Gln Thr Trp Asp Trp Thr Gln Val Asn Ser Val Glu Asp
        675                 680                 685
Leu Val Asp Asn Ala Asp Ile Ala Lys Met Ala Val Val His Lys Ser
    690                 695                 700
Leu Glu Leu Arg Ala Glu Phe Arg Ala Ser Phe Val Gly Gly Asp His
705                 710                 715                 720
Gln Ala Val Phe Gly Glu Gly Arg Ala Glu Ser His Ile Met Gly Ile
                725                 730                 735
Ala Arg Gly Thr Asp Arg Asn His Leu Asn Ile Ile Ala Leu Ala Thr
            740                 745                 750
Arg Arg Pro Leu Ile Leu Glu Asp Arg Gly Gly Trp Tyr Asp Thr Thr
        755                 760                 765
Val Thr Leu Pro Gly Gly Gln Trp Glu Asp Arg Leu Thr Gly Gln Arg
    770                 775                 780
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BNSDOCID: <EP 1174508A2 I >

Phe Ser Gly Val Val Pro Ala Thr Asp Leu Phe Ser His Leu Pro Val
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Ser Leu Leu Val Leu Val Pro Asp Ser Glu Phe
805 810

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<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for PCR

<400> 33
ccaaaatcga taacatcaat cgagatcggg 30

<210> 34
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer for PCR

<400> 34
cttgatcgat taaaaacgct cgacgagccg 30

Claims

1. A coryneform bacterium having L-glutamic acid producing ability, wherein trehalose synthesis ability is decreased or deleted in the bacterium.

2. The coryneform bacteria according to claim 1, wherein the trehalose synthesis ability is decreased or deleted by introducing a mutation into a chromosomal gene coding for an enzyme in trehalose systhesis pathway or disrupting the gene.

3. The coryneform bacteria according to claim 2, wherein the gene coding for the enzyme in trehalose synthesis pathway consists of a gene coding for trehalose-6-phosphate synthase, a gene coding for maltooligosyltrehalose synthase, or both of these genes.

4. The coryneform bacteria according to claim 3, wherein the gene coding for trehalose-6-phosphate synthase codes for the amino acid sequence of SEQ ID NO: 30, and the gene coding for maltooligosyltrehalose synthase codes

for the amino acid sequence of SEQ ID NO: 32.

5. A method for producing L-glutamic acid comprising the steps of culturing a coryneform bacterium according to any one of claims 1-4 in a medium to produce and accumulate L-glutamic acid in the medium, and collecting the L-glutamic acid from the medium.

6. A DNA coding for a protein defined in the following (A) or (B):

(A) a protein having the amino acid sequence of SEQ ID NO: 30,
(B) a protein having the amino acid sequence of SEQ ID NO: 30 including substitution, deletion, insertion or addition of one or several amino acid residues and having trehalose-6-phosphate synthase activity.

7. A DNA according to claim 6, which is a DNA defined in the following (a) or (b):

(a) a DNA containing a nucleotide sequence comprising at least the residues of nucleotide numbers 484-1938 in the nucleotide sequence of SEQ ID NO: 29,
(b) a DNA hybridizable with a nucleotide sequence comprising at least the residues of nucleotide numbers 484-1938 in the nucleotide sequence of SEQ ID NO: 29 under a stringent condition, showing homology of 55% or more to the foregoing nucleotide sequence, and coding for a protein having trehalose-6-phosphate synthase activity.

8. A DNA coding for a protein defined in the following (A) or (B):

(A) a protein having the amino acid sequence of SEQ ID NO: 32,
(B) a protein having the amino acid sequence of SEQ ID NO: 32 including substitution, deletion, insertion or addition of one or several amino acid residues and having maltooligosyltrehalose synthase activity.

9. A DNA according to claim 8, which is a DNA defined in the following (a) or (b):

(a) a DNA containing a nucleotide sequence comprising at least the residues of nucleotide numbers 82-2514 in the nucleotide sequence of SEQ ID NO: 31,
(b) a DNA hybridizable with a nucleotide sequence comprising at least the residues of nucleotide numbers 82-2514 in the nucleotide sequence of SEQ ID NO: 31 under a stringent condition, showing homology of 60% or more to the foregoing nucleotide sequence, and coding for a protein having maltooligosyltrehalose synthase activity.